A THESIS FOR THE DEGREE OF MASTER

Fibroblast growth factor-1 as a mediator of paracrine effects of canine adipose tissue-derived mesenchymal stem cells on *in vitro*-induced insulin resistance models

In vitro 인슐린 저항성 모델에 대한 개 지방유래중간엽줄기세포 paracrine 효과의 매개체 fibroblast growth factor-1

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김현진

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이 논문을 수의학석사학위논문으로 제출함.

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Fibroblast growth factor-1 as a mediator of paracrine effects of canine adipose tissue-derived mesenchymal stem cells on *in vitro*-induced insulin resistance models

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Abstract

In the field of diabetes research, many studies on cell therapy have been conducted using mesenchymal stem cells. This research was intended to shed light on the influence of canine adipose-tissue-derived mesenchymal stem cell conditioned medium (cAT-MSC CM) on *in vitro* insulin resistance models that were induced in differentiated 3T3-L1 adipocytes and the possible mechanisms involved in the phenomenon. Gene expression levels of insulin receptor substrate-1 (IRS-1) and glucose transporter type 4 (GLUT4) were used as indicators of insulin resistance. Relative protein expression levels of IRS-1 and

GLUT4 were augmented in the cAT-MSC CM treatment group compared to insulin resistance model controls, indicating beneficial effects of cAT-MSC to DM, probably by actions of secreting factors. With reference to previous studies on fibroblast growth factor-1 (FGF1), it was proposed as a contributing factor to the mechanism of action. Anti-FGF1 neutralizing antibody was added to the CM-treated insulin resistance models. As a result, significantly diminished protein levels of IRS-1 and GLUT4 were observed, supporting our assumption. Accordingly, this study advocated the potential of MSC CM as an alternative insulin sensitizer and discovered a signalling factor associated with the paracrine effects of cAT-MSC.

Key words: fibroblast growth factor-1; canine adipose tissue-derived mesenchymal stem cells; conditioned medium; diabetes mellitus; insulin resistance; in vitro; 3T3-L1

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1. Introduction

Diabetes mellitus (DM) is an important endocrine disease that accounts for a serious proportion of small animal medicine. In dogs, most DM patients are known to be type 1 DM (T1DM), which is usually well managed by exogenous insulin supplements. However, canine DM with insulin resistance, 'poorly controlled T1DM', not only needs expensive treatment, but also has poor prognosis. In human medicine, several reports advocate that concurrent diseases such as obesity and inflammatory diseases are associated with insulin resistance in DM patients [7, 9, 38, 48]. A study in dogs also asserts the obesity is a cause of insulin resistance [13, 14]. However, the exact pathophysiology of insulin resistance development is not fully understood. Although insulin sensitizers could be an option for the treatment of uncontrolled T1DM, their possible adverse effects such as weight gain, bone loss, and congestive heart failure encourage further effort to develop new drugs for insulin resistance [28].

Mesenchymal stem cells (MSCs) are multipotent stromal cells that have immunomodulatory and regenerative effects. Because they are relatively free from ethical issues, their therapeutic uses for various diseases including DM have been studied globally. Many studies have shown that they have advantageous effects in *in vivo* experiments using diabetic rodent models [3,

6, 11, 16, 23, 25]. Until now, the known mechanisms of MSC actions when applied to DM are as follows: to differentiate directly into insulin-producing cells (IPCs), to regulate the immune system, and to secrete beneficial cytokines and growth factors [50]. In particular, the paracrine effects of MSCs are thought to enhance insulin sensitivity [40].

Fibroblast growth factor-1 (FGF1) which is a member of the FGF family has been known to play crucial role in glucose homeostasis [31]. Perry et al. reported that FGF1 and FGF19 improved glucose metabolism via down regulation of the hypothalamic-pituitary-adrenal axis [33]. It has been documented that exogenous recombinant FGF1 (rFGF1) improved insulin sensitivity as well as normalized blood glucose levels in diabetic mice models [42]. Recently, FGF1 is getting attention as a leading candidate of novel insulin sensitizer.

This study was designed to investigate the effects of canine adipose tissue-derived MSC-conditioned medium (cAT-MSC CM) on an *in vitro* induced insulin resistance model. To explore this, gene expression of markers related to glucose uptake were evaluated and the specific effective factors were discovered.

2. Material and Methods

2.1. Cell culture and characterization

Adipose tissue was obtained from a healthy dog (1-year-old) during ovariohysterectomy at the Seoul National University Veterinary Medicine Teaching Hospital (SNU VMTH). The owner was provided an informed, written consent for research use. The procedure was also approved by the Institutional Animal Care and Use Committee (IACUC) of SNU and the protocol was performed in accordance with the approved guidelines. Canine adipose tissue-derived mesenchymal stem cells (cAT-MSCs) were isolated and cultured as previously described [26]. Before their use in this study, cells were characterized by their ability to express several stem cell markers using flow cytometry. The following antibodies were used: cluster of differentiation (CD) 29-Fluorescein isothiocyanate (FITC), CD31-FITC, CD34-phycoerythrin (PE), CD73-PE (BD Biosciences, Franklin Lakes, NJ, USA), CD44-FITC, CD45-FITC, and CD90-allophycocyanin (APC) (eBiosciences, San Diego, CA, USA)-conjugated antibodies. Cells were analysed by a BD FACSAria II system (BD Biosciences). Cellular differentiation was confirmed using PRIME-XV® Chondrogenic Differentiation Xeno-Free Serum-Free Medium (XSFM), PRIME-XV® Osteogenic Differentiation Serum-Free Medium (SFM), and PRIME-XV®

Adipogenic Differentiation SFM (all from Irvine Scientific, Santa Ana, CA, USA) according to the manufacturer's instructions followed by Alcian Blue staining, Alizarin Red staining, and Oil Red O staining, respectively.

2.2. In vitro cellular insulin resistance models

The *in vitro* induced insulin resistance model was developed in differentiated 3T3-L1 adipocytes. Murine 3T3-L1 cells were purchased from the Korean Cell Line Bank (Seoul, Korea). 3T3-L1 preadipocytes were differentiated using 3T3-L1 Differentiation Kit (Sigma-Aldrich) and all the differentiation procedures performed following the manufacturer's instructions. Once the proportion of differentiated cells reached 70%, induction of insulin resistance was initiated. Cells were washed with phosphate-buffered saline (PBS; PAN Biotech, Aidenbach, Germany) and changed to serum-free Dulbecco's modified Eagle's medium: Nutrient Mixture F-12 (1:1) (DMEM / F12 (1:1); PAN Biotech). Insulin resistance was induced with treatment with both 40 ng / mL of tumour necrosis factor-α (TNF-α; PeproTech, Rocky Hill, NJ, USA) and 1% oxygen for 24 h. For hypoxic incubation, cells were placed in a hypoxic incubator (ViVAGEN, Sungnam, Korea) with the conditions of 1% O2 at 37 °C.

2.3. Preparation of cAT-MSC-conditioned medium (CM), and anti-fibroblast growth factor-1 (FGF1) antibody treatment

cAT-MSCs (3 x 10^5 cells / well) were seeded in 6-well plates and cultured in 3 mL of DMEM medium containing 10% fetal bovine serum (FBS; PAN Biotech) for 3 days, yielding conditioned medium. After 3 days, conditioned medium was harvested and centrifuged at 850 rpm for 3 min to remove cellular debris. After centrifugation, the supernatant was transferred to a conical tube and stored at -80 °C until use. Anti-FGF1 neutralizing antibody (Abcam, Cambridge, MA, USA) was added to final concentrations of 0.9 μg / ml of culture medium.

2.4. RNA extraction, cDNA synthesis, and quantitative real-time PCR

Total RNAs from all cell groups were extracted using the Easy-BLUE Total RNA Extraction kit (Intron Biotechnology, Sungnam, Korea). The concentration and purity of RNA samples were measured by a spectrophotometer (Implen, Munich, Germany). First strand cDNA was synthesized using LaboPass M-MuLV Reverse Transcriptase (Cosmo

Genetech, Seoul, Korea) according to the manufacturer's instructions. Quantitative real-time (qRT)-PCR analysis was performed in duplicate in 96-well plates with StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using 10 μL of AMPIGENE qPCR Green Mix Hi-ROX with SYBR Green dye (Enzo Life Sciences, Farmingdale, NY, USA) and 400 nM forward and reverse primers (Cosmo Genetech). The cycling conditions were as follows: polymerase activation for 2 minutes at 95 °C, followed by 40 cycles of denaturation for 5 seconds at 95 °C and annealing for 25 seconds at 60 °C. Expression levels were normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

2.5. Western blot analysis

Total proteins were extracted from all cell groups using PRO-PREP Protein Extraction Solution (Intron Biotechnology) according to the manufacturer's instructions. The concentrations of the protein samples were measured using the Bio-Rad DC Protein Assay Kit (Bio-Rad, Hercules, CA, USA). The proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked by 5% non-fat dry milk in Tris-buffered saline containing 0.1%

Tween 20 and incubated with primary antibodies against insulin receptor substrate 1 (IRS-1, 1:500; Abcam) and glucose transporter type 4 (GLUT4, 1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C overnight. The membranes were incubated with secondary antibodies at room temperature for 1 h. The immunoreactive bands were visualised using enhanced chemiluminescence (Advansta, Menlo Park, CA, USA) and normalised to β-actin levels (Santa Cruz Biotechnology).

2.6. Statistical analysis

Data are shown as mean ± standard deviation. Statistical comparisons between groups were made with use of one-way ANOVA and an unpaired Student's t test using the GraphPad Prism v.6.01 software (GraphPad Inc., La Jolla, CA, USA). P value of < 0.05 was considered statistically significant.

3. Results

3.1. Characterization of cAT-MSCs

Cells obtained from canine adipose tissue were characterized by their ability to express stem cell markers and to differentiate toward adipogenic, osteogenic, and chondrogenic lineages when cultured in media containing lineage-specific factors. The known MSC markers such as CD29, CD73, CD44, and CD90 were highly expressed by the cells. Negative markers such as CD31, CD34, and CD45 were not expressed (Figure. 1a). The multi-lineage plasticity of cAT-MSCs was confirmed by specific staining methods: Oil Red O staining, Alizarin Red S staining, and Alcian Blue staining, respectively (Figure. 1b).

3.2. Differentiation into adipocytes and development of an insulin resistance model in 3T3-L1 adipocytes

All processes of differentiation and induction of insulin resistance in the 3T3-L1 are depicted schematically in Figure 2a. During differentiation, 3T3-L1 preadipocytes transformed from a fibroblast-like appearance to an adipocyte-like appearance and cytoplasmic lipid droplet accumulation

became remarkable (Figure. 2b-d). After over 70% of 3T3-L1 cells differentiated, cells were treated with TNF-α and incubated in hypoxic conditions for 24 h to induce insulin resistance. The mRNA expression levels of IRS-1 and GLUT4 were compared as markers of insulin resistance. As a result, both IRS-1 and GLUT4 mRNA levels showed similar tendencies through the differentiation to insulin resistance induction, but the degree of alterations was greater in GLUT4. The mRNA expression levels of insulin resistance markers were remarkably increased during the differentiation, while they were markedly decreased in the insulin resistance model (Figure. 2e-f).

3.3. Effects of cAT-MSC CM treatment on IRS-1 and GLUT4 expressions in the insulin resistance model

To evaluate the therapeutic effects of cAT-MSC CM on the insulin resistance model, expression levels of IRS-1 and GLUT4 were examined. In the perspective of IRS-1 expression levels, mRNA levels were not changed significantly. However, the protein levels of IRS-1 revealed significant elevation. In GLUT4 expression, both mRNA and protein levels were significantly increased after cAT-MSC CM treatment (Figure. 3).

3.4. Reduced therapeutic effects of cAT-MSC CM with anti-FGF1 neutralizing antibody treatment

FGF1 in the cAT-MSC CM suspected to play an important role in the alterations in the expression levels of IRS-1 and GLUT4. To verify the hypothesis, an anti-FGF1 neutralizing antibody was added to the cAT-MSC CM treated insulin resistance model. By measuring the protein levels of insulin resistance markers, data confirmed that the improvement of their expression levels was significantly reduced (Figure. 4).

4. Discussion

There have been doubts that exogenous insulin supplements for poorly controlled T1DM or T2DM were not enough to maintain the patient in a good condition. In this regard, researches into cell therapy using MSCs have been actively conducted. Mechanisms of their therapeutic effects to DM may be explained through various molecular processes. MSCs have the ability to directly differentiate into IPCs [18, 24, 46] and regulate the immune system to protect pancreatic islet cells from further destruction [1, 8, 17, 22, 51]. In addition, they possess powerful paracrine effects via secretion of a variety of cytokines and growth factors, enabling them to have anti-inflammatory effects and regulate insulin signalling and resulting in the improvement of insulin sensitivity [10, 32, 40, 47]. Several insulin sensitizers (such as biguanides and thiazolidinediones) were considered as therapeutics for DM with insulin resistance. However, long-term medication with these drugs can cause various adverse effects, which highlights the necessity for a new strategy.

In these experiments, 3T3-L1 adipocytes were used to develop an *in vitro* insulin resistance model. There have been diverse studies into inducing insulin resistance in 3T3-L1 cell lines. For example, various agents including TNF-α, Interleukin-1 (IL-1), IL-6, free fatty acids, dexamethasone,

and high insulin were used to make models of insulin resistance in 3T3-L1 adipocytes [20, 30, 35-37, 43]. Lo et al. compared transcriptome analysis data of *in vitro* insulin resistance models and *in vivo* diet-induced obese mouse models. Considering their finding that the TNF-α and hypoxia co-treatment model most closely resembled *in vivo* alterations [29], the model was employed in this experiment. For the purpose of monitoring insulin resistance, IRS-1 and GLUT4 were selected as markers of glucose uptake [41, 49]. IRS-1 is a signalling protein that plays an important role at the initial part of insulin signalling pathway [15]. GLUT4 is a transporter protein that is mainly expressed in the skeletal muscle, adipose tissue, and heart [19]. Its up regulation and translocation to the cell membranes are thought to be critical steps of the insulin responsive increment of glucose uptake [34].

According to early studies, the effectiveness of MSC in DM originated from the potential to trans-differentiation towards IPCs [4, 45]. However, these theories were unable to give a good explanation for the disappointing results of *in vivo* cell tracking studies [5, 27]. Recent studies suggested that the secretory capacity of MSCs would play a crucial role for the therapeutic effects. Gao et al. isolated and injured pancreatic islets *in vitro* and then assessed islet regeneration after the treatment with MSC CM. They found that both β cell replication and islet progenitor differentiation were

promoted and the PI3K / Akt signal pathway was involved [12]. Another study revealed that AT-MSC CM enhanced glucose uptake in 3T3-L1 and C2C12 insulin resistance models [39]. cAT-MSC CM was also utilized to explore the paracrine effect of cAT-MSC in our experiments.

FGF family is a signalling protein group composed of 18 members mediating a variety of biological functions in cell proliferation and developmental processes. Each family member performs unique actions by binding to FGF receptors, which results in activation of intracellular downstream cascades [2]. FGF1, identified as a regulator of adipose tissue remodelling and metabolic homeostasis, is a downstream molecule of peroxisome proliferator-activated receptor-γ (PPAR-γ), which is a target of the thiazolidinedione class of insulin sensitizers [21]. It has been established that rFGF1 injection normalized glucose levels and enhanced insulin sensitivity in diabetic mice. What was noteworthy is that the effect was insulin-dependent. Moreover, rFGF1 did not induce side effects that could be incurred by chronic use of traditional insulin sensitizers. Furthermore, genetically modified rFGF1 lacking the mitogen properties of rFGF1 had similar glucose lowering effects to some extent [42], intensifying the possibility of rFGF1 as a substitute of conventional insulin sensitizers. Based on the fact that MSC CM contains considerable amounts of FGF1

[44], I attempted to find out whether FGF1 contributed to the advantageous effects of cAT-MSC CM on insulin resistant models.

In the present study, gene expression levels of IRS-1 and GLUT4, which were considered as markers of insulin responsive glucose uptake, were evaluated. It was shown that reduced expression levels of these markers in 3T3-L1 insulin resistance models were restored after the treatment of cAT-MSC CM. This finding was consistent with previous research that suggested that MSC could improve insulin resistance through the paracrine signalling of various cytokines and growth factors [39]. Since FGF1 was suspected to have a role in this effect, an anti-FGF1 neutralizing antibody was added into CM-treated 3T3-L1 insulin resistance models and assessed alterations of gene expression levels. The anti-FGF1 antibody group represented diminished insulin resistance marker gene expression levels in comparison to the CM-treated group. This information led to the inference that FGF1 included in cAT-MSC CM is one of the mediators of the signalling pathways that induces therapeutic effects. Although these experiments could not exclude the possibility that various cytokines and growth factors abundantly contained in cAT-MSC CM (other than FGF1) could affect the alterations in expression levels, it is worth mentioning that this study is the first work that revealed FGF1 as a specific mediator of insulin-sensitizing effects of cAT-MSC CM in DM.

4. Conclusion

This study identified that the induced insulin resistance in 3T3-L1 cells was ameliorated in the presence of cAT-MSC CM by measuring protein expression levels of IRS-1 and GLUT4. Additional data showed that the improvement was inhibited by the addition of anti-FGF1 neutralizing antibody, suggesting that FGF1 would act as a mediator of the beneficial effects of cAT-MSC CM. It is noteworthy that the present study is the first to reveal a specific functioning component of MSC CM in *in vitro* insulin resistance models, and further studies to find out other effective factors of MSC CM will be necessary.

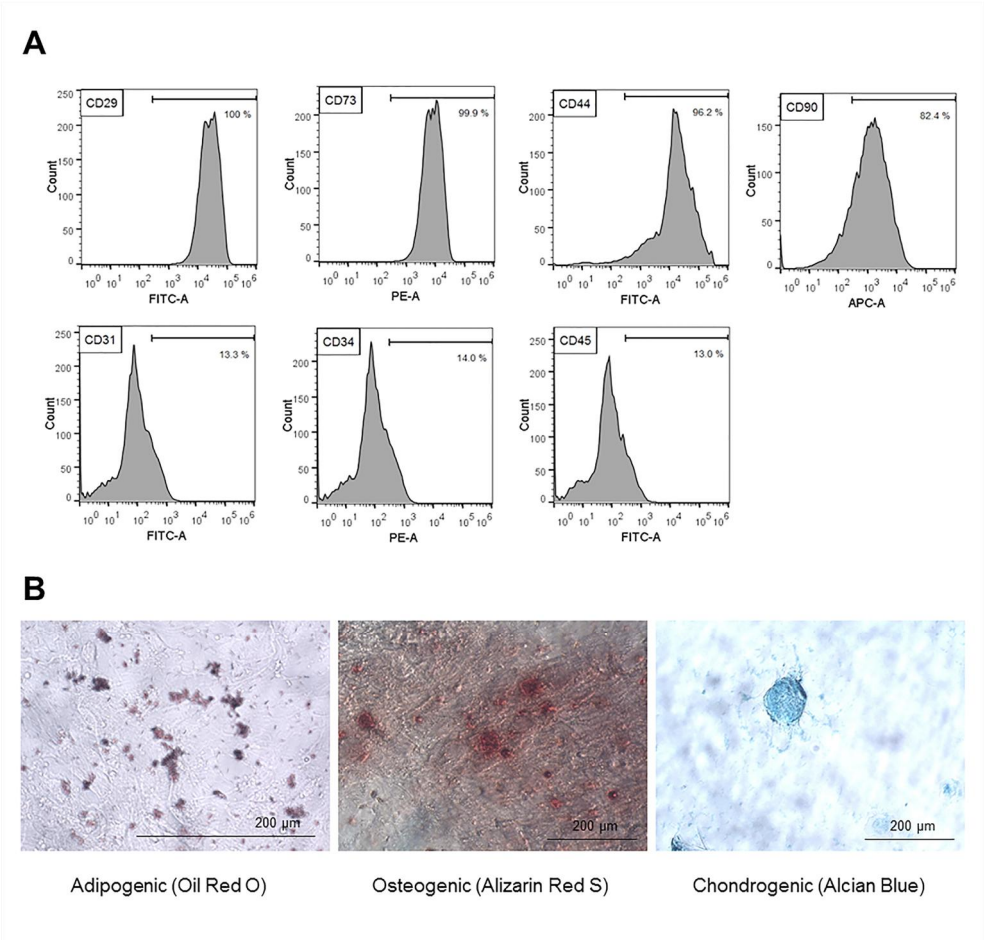

Figure 1. Flow cytometry and special staining characterized cAT-MSCs. (A) Immunophenotypic analysis was performed by flow cytometry using positive markers CD29, CD73, CD44, and CD90 and negative markers CD31, CD34, and CD45. (B) Adipogenic (Oil Red O staining), osteogenic (Alizarin Red S staining), and chondrogenic (Alcian Blue staining) differentiation abilities of cAT-MSCs (from left to right) were confirmed. Original magnification: 400x (left), 200x (middle, right).

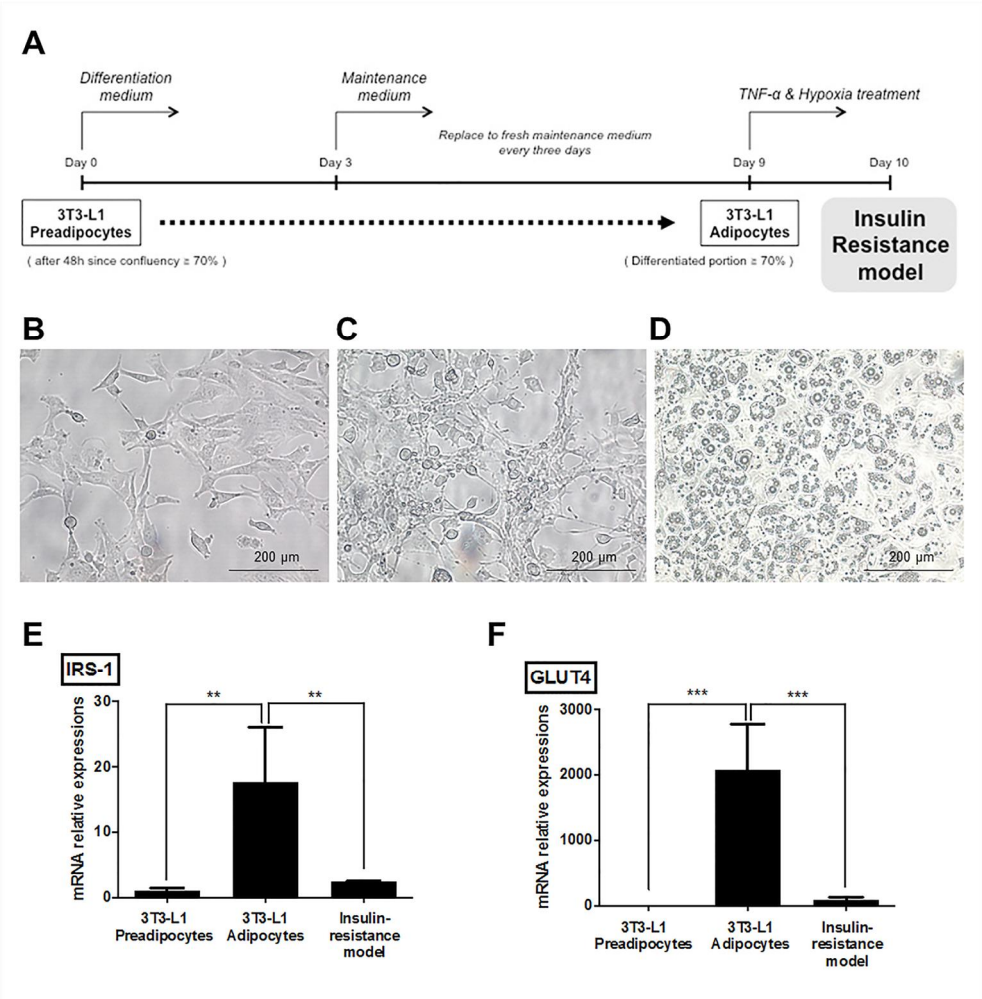

Figure 2. Insulin resistance was induced in 3T3-L1 adipocytes *in vitro*. (A) Adipocyte differentiation of 3T3-L1 cells and following induction of insulin resistance were summarized with the course of time. (B) Fibroblast-like 3T3-L1 preadipocytes at day 0. (C) Differentiating 3T3-L1 cells at day 3. (D) Differentiated 3T3-L1 adipocytes containing cytoplasmic lipid droplets at day 9. Original magnification: 200x. (E, F) Alterations in mRNA

expression levels of IRS-1 and GLUT4 during the differentiation and insulin resistance induction were shown. Data are shown as mean ± standard deviation. **P < 0.01, ***P < 0.001.

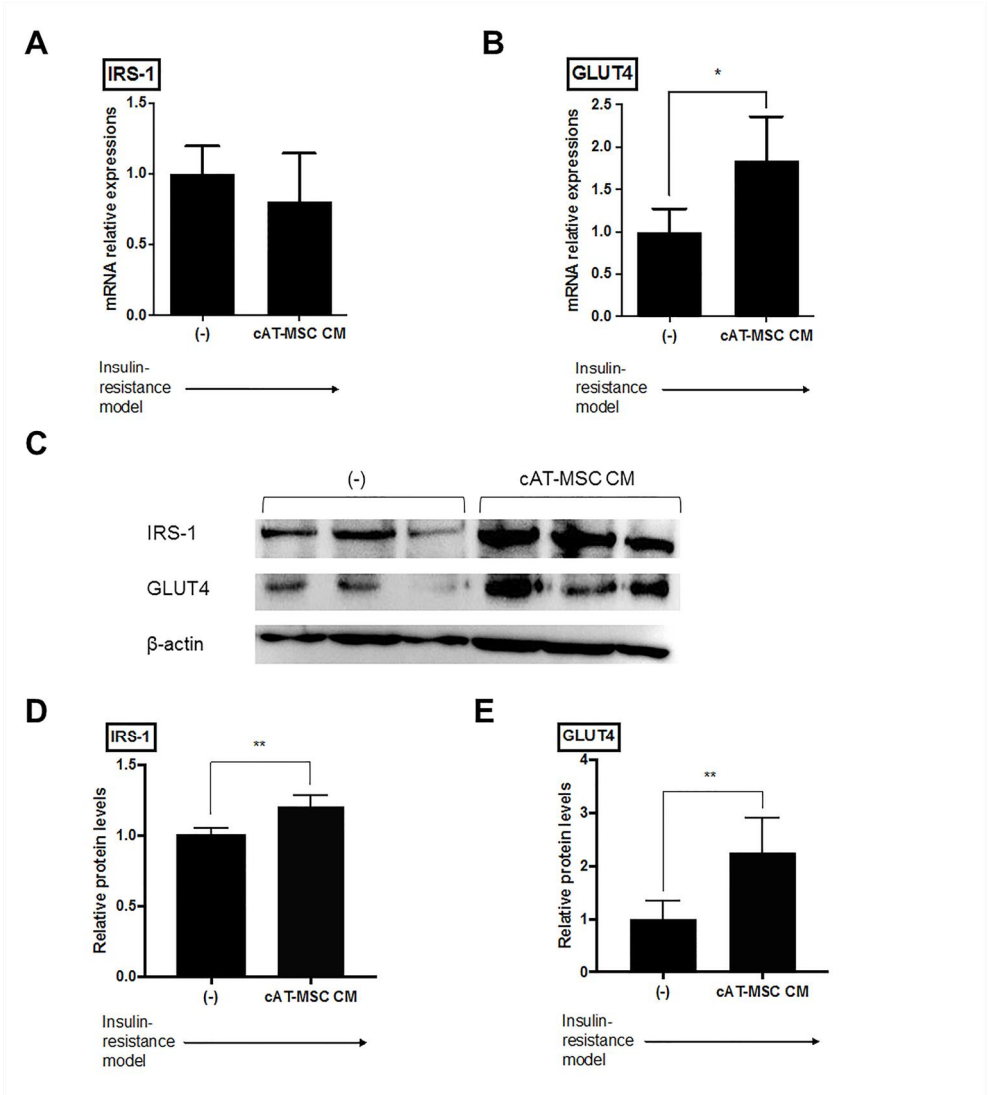

Figure 3. Treatment of cAT-MSC CM affected gene expressions of IRS-1 and GLUT4 in 3T3-L1 insulin resistance models. (A) The mRNA expression levels of IRS-1 were not significantly changed. (B) The mRNA expression levels of GLUT4 showed a significant elevation. (C) Western blot. (-): insulin resistance models of 3T3-L1 cells, cAT-MSC CM: cAT-

MSC CM-treated insulin resistance models of 3T3-L1 cells. (D-E) Up regulation of IRS-1 and GLUT4 gene expression was observed in CM-treated insulin resistance models. Data are shown as mean ± standard deviation. *P < 0.05, **P < 0.01.

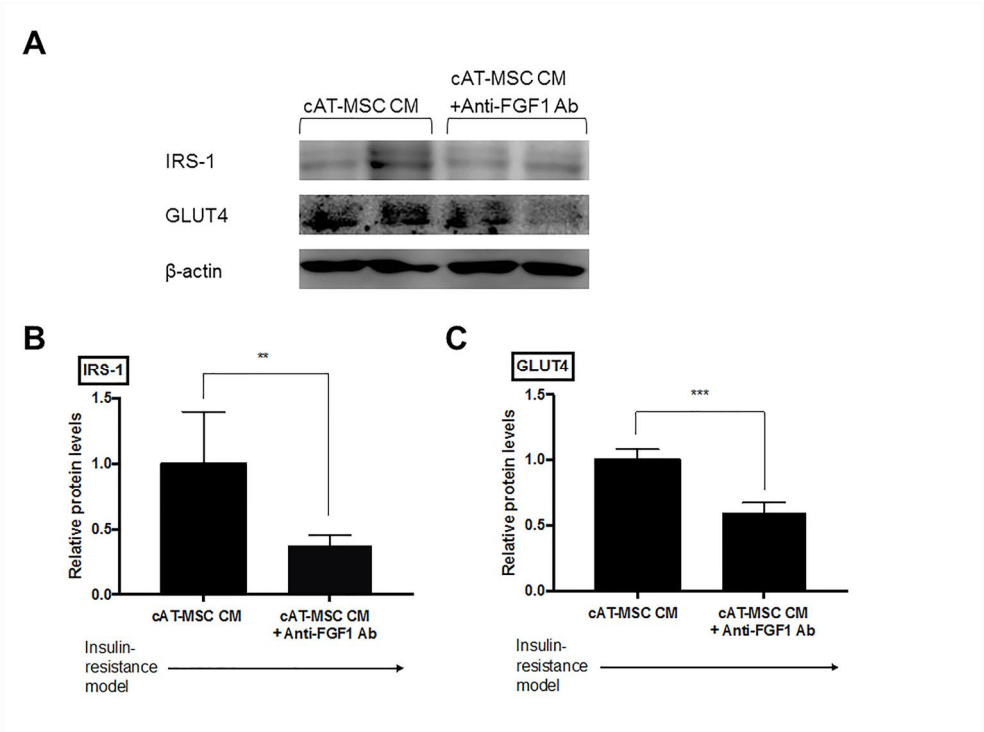

Figure 4. Gene expressions of IRS-1 and GLUT4 were down regulated after blocking of FGF1 function. (A) Western blot. (B-C) Relative protein levels of IRS-1 and GLUT4 were lower in the insulin resistance models treated with both cAT-MSC CM and anti-FGF1 antibody than the models treated with cAT-MSC CM alone. Data are shown as mean ± standard deviation. **P < 0.01, ***P < 0.001.

References

1. **Bachar-Wikstrom E, Wikstrom JD, Ariav Y, Tirosh B, Kaiser N, Cerasi E, and Leibowitz G.** Stimulation of autophagy improves endoplasmic reticulum stress–induced diabetes. Diabetes 2013, **62**, 1227-1237.

2. **Beenken A, and Mohammadi M.** The FGF family: biology, pathophysiology and therapy. Nature Reviews Drug Discovery 2009, **8**, 235.

3. **Bhansali A, Upreti V, Khandelwal N, Marwaha N, Gupta V, Sachdeva N, Sharma R, Saluja K, Dutta P, and Walia R.** Efficacy of autologous bone marrow–derived stem cell transplantation in patients with type 2 diabetes mellitus. Stem Cells And Development 2009, **18**, 1407-1416.

4. **Chandra V, Phadnis S, Nair PD, and Bhonde RR.** Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells. Stem Cells 2009, **27**, 1941-1953.

5. **Choi J, Uchino H, Azuma K, Iwashita N, Tanaka Y, Mochizuki H, Migita M, Shimada T, Kawamori R, and Watada H.** Little evidence of transdifferentiation of bone marrow-derived cells into pancreatic beta cells. Diabetologia 2003, **46**, 1366-1374.

6. **Czubak P, Bojarska-Junak A, Tabarkiewicz J, and Putowski L.** A modified method of insulin producing cells' generation from bone marrow-derived mesenchymal stem cells. Journal Of Diabetes Research 2014, **2014**.

7. **Dandona P, Aljada A, and Bandyopadhyay A.** Inflammation: the link between insulin resistance, obesity and diabetes. Trends in Immunology 2004, **25**, 4-7.

8. **Ebato C, Uchida T, Arakawa M, Komatsu M, Ueno T, Komiya K, Azuma K, Hirose T, Tanaka K, and Kominami E.** Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet. Cell Metabolism 2008, **8**, 325-332.

9. **Festa A, D'agostino R, Howard G, Mykkänen L, Tracy RP, and Haffner SM.** Chronicsubclinical inflammation as part of the insulin resistance syndrome. Circulation 2000, **102**, 42-47.

10. **Fujisaka S, Usui I, Bukhari A, Ikutani M, Oya T, Kanatani Y, Tsuneyama K, Nagai Y, Takatsu K, and Urakaze M.** Regulatory mechanisms for adipose tissue M1 and M2 macrophages in diet-induced obese mice. Diabetes 2009, **58**, 2574-2582.

11. **Gabr MM, Zakaria MM, Refaie AF, Khater SM, Ashamallah SA, Ismail AM, El-Halawani SM, and Ghoneim MA.** Differentiation of

human bone marrow-derived mesenchymal stem cells into insulin-producing cells: evidence for further maturation in vivo. BioMed Research International 2015, **2015**.

12. **Gao X, Song L, Shen K, Wang H, Qian M, Niu W, and Qin X.** Bone marrow mesenchymal stem cells promote the repair of islets from diabetic mice through paracrine actions. Molecular and Cellular Endocrinology 2014, **388**, 41-50.

13. **German AJ.** The growing problem of obesity in dogs and cats. The Journal of Nutrition 2006, **136**, 1940S-1946S.

14. **German AJ, Hervera M, Hunter L, Holden SL, Morris PJ, Biourge V, and Trayhurn P.** Improvement in insulin resistance and reduction in plasma inflammatory adipokines after weight loss in obese dogs. Domestic Animal Endocrinology 2009, **37**, 214-226.

15. **Gual P, Le Marchand-Brustel Y, and Tanti J-F.** Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. Biochimie 2005, **87**, 99-109.

16. **Hao H, Liu J, Shen J, Zhao Y, Liu H, Hou Q, Tong C, Ti D, Dong L, and Cheng Y.** Multiple intravenous infusions of bone marrow mesenchymal stem cells reverse hyperglycemia in experimental type 2 diabetes rats. Biochemical and Biophysical Research Communications

2013, **436**, 418-423.

17. **Hematti P, Kim J, Stein AP, and Kaufman D.** Potential role of mesenchymal stromal cells in pancreatic islet transplantation. Transplantation Reviews 2013, **27**, 21-29.

18. **Hess D, Li L, Martin M, Sakano S, Hill D, Strutt B, Thyssen S, Gray DA, and Bhatia M.** Bone marrow-derived stem cells initiate pancreatic regeneration. Nature Biotechnology 2003, **21**, 763.

19. **Im SS, Kwon SK, Kim TH, Kim Hi, and Ahn YH.** Regulation of glucose transporter type 4 isoform gene expression in muscle and adipocytes. International Union of Biochemistry and Molecular Biology Life 2007, **59**, 134-145.

20. **Jager J, Grémeaux T, Cormont M, Le Marchand-Brustel Y, and Tanti J-F.** Interleukin-1β-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. Endocrinology 2007, **148**, 241-251.

21. **Jonker JW, Suh JM, Atkins AR, Ahmadian M, Li P, Whyte J, He M, Juguilon H, Yin YQ, Phillips CT, Yu RT, Olefsky JM, Henry RR, Downes M, and Evans RM.** A PPARγ-FGF1 axis is required for adaptive adipose remodelling and metabolic homeostasis. Nature 2012, **485**, 391-394.

22. **Jung HS, Chung KW, Kim JW, Kim J, Komatsu M, Tanaka K, Nguyen YH, Kang TM, Yoon K-H, and Kim J-W.** Loss of autophagy diminishes pancreatic β cell mass and function with resultant hyperglycemia. Cell Metabolism 2008, **8**, 318-324.

23. **Kadam S, Govindasamy V, and Bhonde R.** Generation of functional islets from human umbilical cord and placenta derived mesenchymal stem cells. Somatic Stem Cells: Methods and Protocols 2012, 291-313.

24. **Kadam S, Muthyala S, Nair P, and Bhonde R.** Human placenta-derived mesenchymal stem cells and islet-like cell clusters generated from these cells as a novel source for stem cell therapy in diabetes. The Review Of Diabetic Studies: RDS 2010, **7**, 168.

25. **Kadam SS, and Bhonde RR.** Islet neogenesis from the constitutively nestin expressing human umbilical cord matrix derived mesenchymal stem cells. Islets 2010, **2**, 112-120.

26. **Kim H-W, Song W-J, Li Q, Han S-M, Jeon K-O, Park S-C, Ryu M-O, Chae H-K, Kyeong K, and Youn H-Y.** Canine adipose tissue-derived mesenchymal stem cells ameliorate severe acute pancreatitis by regulating T cells in rats. Journal of Veterinary Science 2016, **17**, 539-548.

27. **Lechner A, Yang Y-G, Blacken RA, Wang L, Nolan AL, and Habener JF.** No evidence for significant transdifferentiation of bone marrow into

pancreatic β-cells in vivo. Diabetes 2004, **53**, 616-623.

28. **Lehrke M, and Lazar MA.** The Many Faces of PPARγ. Cell 2005, **123**, 993-999.

29. **Lo K, Labadorf A, Kennedy NJ, Han M, Yap Y, Matthews B, Xin X, Sun L, Davis RJ, Lodish HF, and Fraenkel E.** Analysis of in vitro insulin-resistance models and their physiological relevance to invivo diet-induced adipose insulin resistance. Cell Reports 2013, **5**, 259-270.

30. **Nguyen MA, Satoh H, Favelyukis S, Babendure JL, Imamura T, Sbodio JI, Zalevsky J, Dahiyat BI, Chi N-W, and Olefsky JM.** JNK and tumor necrosis factor-α mediate free fatty acid-induced insulin resistance in 3T3-L1 adipocytes. Journal of Biological Chemistry 2005, **280**, 35361-35371.

31. **Nies VJM, Sancar G, Liu W, Van Zutphen T, Struik D, Yu RT, Atkins AR, Evans RM, Jonker JW, and Downes MR.** Fibroblast growth factor signaling in metabolic regulation. Frontiers in Endocrinology 2016, **6**.

32. **Olefsky JM, and Glass CK.** Macrophages, inflammation, and insulin resistance. Annual Review of Physiology 2010, **72**, 219-246.

33. **Perry RJ, Lee S, Ma L, Zhang D, Schlessinger J, and Shulman GI.** FGF1 and FGF19 reverse diabetes by suppression of the hypothalamic-pituitary-adrenal axis. Nature Communications 2015, **6**.

34. **Pessin JE, and Saltiel AR.** Signaling pathways in insulin action: molecular targets of insulin resistance. Journal of Clinical Investigation 2000, **106**, 165.

35. **Rotter V, Nagaev I, and Smith U.** Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-α, overexpressed in human fat cells from insulin-resistant subjects. Journal of Biological Chemistry 2003, **278**, 45777-45784.

36. **Ruan H, Hacohen N, Golub TR, Van Parijs L, and Lodish HF.** Tumor necrosis factor-α suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes: Nuclear factor-κB activation by TNF-α is obligatory. Diabetes 2002, **51**, 1319-1336.

37. **Sakoda H, Ogihara T, Anai M, Funaki M, Inukai K, Katagiri H, Fukushima Y, Onishi Y, Ono H, and Fujishiro M.** Dexamethasone-induced insulin resistance in 3T3-L1 adipocytes is due to inhibition of glucose transport rather than insulin signal transduction. Diabetes 2000, **49**, 1700-1708.

38. **Shoelson SE, Lee J, and Goldfine AB.** Inflammation and insulin resistance. Journal of Clinical Investigation 2006, **116**, 1793.

39. **Shree N, and Bhonde RR.** Conditioned media from adipose tissue derived mesenchymal stem cells reverse insulin resistance in cellular

models. Journal of Cellular Biochemistry 2016.

40. **Si Y, Zhao Y, Hao J, Liu J, Guo Y, Mu Y, Shen J, Cheng Y, Fu X, and Han W.** Infusion of mesenchymal stem cells ameliorates hyperglycemia in type 2 diabetic rats: Identification of a novel role in improving insulin sensitivity. Diabetes 2012, **61**, 1616-1625.

41. **Stephens JM, Lee J, and Pilch PF.** Tumor necrosis factor-α-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. Journal of Biological Chemistry 1997, **272**, 971-976.

42. **Suh JM, Jonker JW, Ahmadian M, Goetz R, Lackey D, Osborn O, Huang Z, Liu W, Yoshihara E, and van Dijk TH.** Endocrinization of FGF1 produces a neomorphic and potent insulin sensitizer. Nature 2014, **513**, 436-439.

43. **Thomson MJ, Williams MG, and Frost SC.** Development of insulin resistance in 3T3-L1 adipocytes. Journal of Biological Chemistry 1997, **272**, 7759-7764.

44. **Wu L, Leijten J, van Blitterswijk CA, and Karperien M.** Fibroblast growth factor-1 is a mesenchymal stromal cell-secreted factor stimulating proliferation of osteoarthritic chondrocytes in co-culture. Stem Cells and

Development 2013, **22**, 2356-2367.

45. **Wu X-H, Liu C-P, Xu K-F, Mao X-D, Zhu J, Jiang J-J, Cui D, Zhang M, Xu Y, and Liu C.** Reversal of hyperglycemia in diabetic rats by portal vein transplantation of islet-like cells generated from bone marrow mesenchymal stem cells. World Journal of Gastroenterology: WJG 2007, **13**, 3342.

46. **Xie Q-P, Huang H, Xu B, Dong X, Gao S-L, Zhang B, and Wu Y-L.** Human bone marrow mesenchymal stem cells differentiate into insulin-producing cells upon microenvironmental manipulation in vitro. Differentiation 2009, **77**, 483-491.

47. **Xie Z, Hao H, Tong C, Cheng Y, Liu J, Pang Y, Si Y, Guo Y, Zang L, and Mu Y.** Human umbilical cord-derived mesenchymal stem cells elicit macrophages into an anti-inflammatory phenotype to alleviate insulin resistance in type 2 diabetic rats. Stem Cells 2016, **34**, 627-639.

48. **Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, and Tartaglia LA.** Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. Journal of Clinical Investigation 2003, **112**, 1821.

49. **Yi P, Lu F-E, Xu L-J, Chen G, Dong H, and Wang K-F.** Berberine reverses free-fatty-acid-induced insulin resistance in 3T3-L1 adipocytes

through targeting IKKβ. World Journal of Gastroenterology: WJG 2008, **14**, 876.

50. **Zang L, Hao H, Liu J, Li Y, Han W, and Mu Y.** Mesenchymal stem cell therapy in type 2 diabetes mellitus. Diabetology & Metabolic Syndrome 2017, **9**, 36.

51. **Zhao K, Hao H, Liu J, Tong C, Cheng Y, Xie Z, Zang L, Mu Y, and Han W.** Bone marrow-derived mesenchymal stem cells ameliorate chronic high glucose-induced β-cell injury through modulation of autophagy. Cell Death & Disease 2015, **6**, e1885.

국문 초록

In vitro 인슐린 저항성 모델에 대한 개 지방유래중간엽줄기세포 paracrine 효과의 매개체 fibroblast growth factor-1

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당뇨 연구 분야에서 중간엽줄기세포를 이용한 세포 치료 연구가 널리 진행되고 있다. 본 연구는 3T3-L1 지방세포에서 유도된 생체 외 인슐린 저항성 모델에서 개 지방유래중간엽줄기세포의 치료 효과를 알아보고, 그 기전을 밝히기 위해 진행되었다. 인슐린 저항성의 지표로서 insulin receptor substrate-1(IRS-1)과 glucose

transporter type 4(GLUT4)의 유전자 발현 수준을 이용하였다. 개 지방유래중간엽줄기세포를 처리한 인슐린 저항성 모델군은 대조군에 비해 IRS-1와 GLUT4의 상대적인 단백질 발현 수준이 증가되었는데, 이는 당뇨에 대한 개 지방유래중간엽줄기세포의 치료 효과가 분비 인자에 의한 것임을 시사하였다. Fibroblast growth factor-1(FGF1)에 대한 선행연구들을 참고하여 FGF1이 이 기전에 기여할 것이라는 가설을 세우고, anti-FGF1 중화 항체를 줄기세포 처리군에 첨가한 결과, IRS-1과 GLUT4의 단백질 발현 수준이 유의적으로 감소하는 것을 볼 수 있었으며, 이는 앞의 가설을 뒷받침하였다. 따라서 본 연구는 중간엽줄기세포가 인슐린 증감제 대체제를 찾기 위한 연구 대상으로서의 가능성을 지닌다는 점을 확인하였고, paracrine 경로로 작용하는 신호 인자를 새롭게 발견하였다.

주요어: fibroblast growth factor-1; 개 지방유래중간엽줄기세포; 당뇨; 인슐린 저항성; 생체 외; 3T3-L1

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